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(54) **DRUG COMBINATIONS FOR REDUCING CELL VIABILITY AND/OR CELL PROLIFERATION**

ARZNEIMITTELKOMBINATIONEN ZUR VERRINGERUNG DER ZELLEBENSFÄHIGKEIT UND/ODER ZELLPROLIFERATION

ASSOCIATIONS MÉDICAMENTEUSES PERMETTANT DE RÉDUIRE LA VIABILITÉ ET/OU LA PROLIFÉRATION CELLULAIRE

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(56) References cited:
WO-A1-2015/057938 US-A1- 2011 179 505

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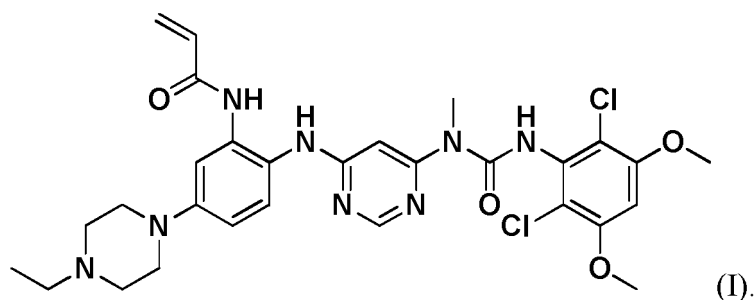
Description

BACKGROUND

5 **[0001]** Liver cancer is the second greatest cause of mortality from any type of cancer, and the 16th most common cause of death worldwide (Llovet JM, et al., 2015 "Advances in targeted therapies for hepatocellular carcinoma in the genomic era." Nat. Rev. Clin Oncology 12, 408-424). Hepatocellular carcinoma (HCC) accounts for up to 90% of all primary liver cancers (Llovet JM et al. 2015).

10 **[0002]** Various signaling pathways have been implicated in HCC, including fibroblast growth factors (FGF) (particularly FGF19/FGFR4), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), ERK/MAPK, and mechanistic target of rapamycin (mTOR), among others (Llovet JM et al., 2015). FGF19 is overexpressed in about a third of all HCC, and this overexpression is hypothesized to hyperactivate FGFR4 and its downstream signaling pathway leading to enhanced tumor growth (Xie MH et al., 1999 "FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4." Cytokine. 1999 Oct;11(10):729-35; Sawey, et al., 2011 "Identification of a therapeutic strategy targeting amplified FGF19 in liver cancer by Oncogenomic screening." Cancer Cell. 2011 Mar 8;19(3):347-58.). Similar to FGF19/FGFR4 pathway, EGFR is also overexpressed in 40-70% of HCC, and its activation is involved in HCC pathogenesis (Chua CWL et al., 2011. Targeted Therapy in Hepatocellular Carcinoma. Int J Hepatol. 2011; 2011: 348297).

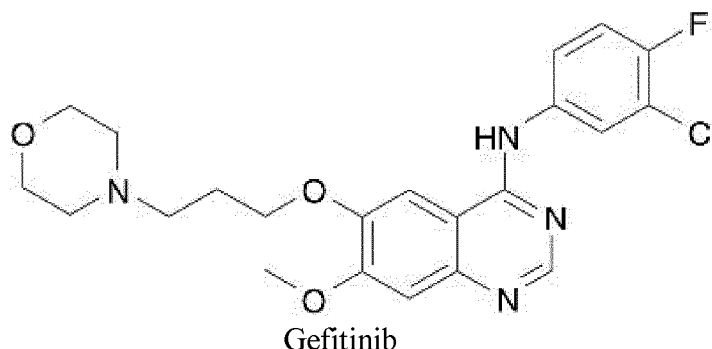
15 **[0003]** Compound 1 is a selective, orally bioavailable small molecule FGFR4 inhibitor with the structure shown in Formula I, and the chemical name N-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide:



35 **[0004]** Compound 1 and its synthesis are reported in PCT International Application Publication No. WO2015/057938, published on April 23, 2015.

[0005] In US 2011/0179505 A1, a rodent animal model was chosen for studying the molecular mechanisms and physiological processes associated with uncontrolled cell growth as observed in cancer using a modified FGFR4 receptor.

[0006] Gefitinib (4-quinazolinamine N-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy]) is an FDA-approved EGFR inhibitor. Gefitinib is a free base with the following structure:



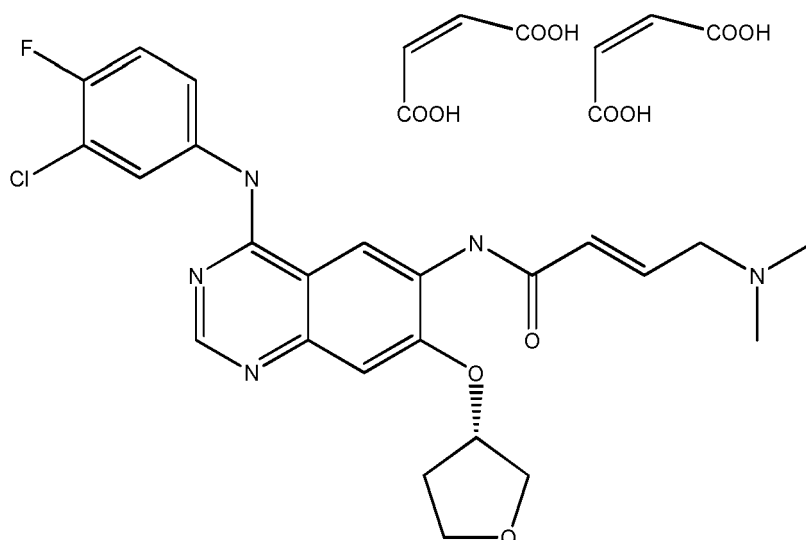
[0007] See U.S. Patent No. 5,770,599.

55 **[0008]** Afatinib (2-butenamide, N-[4-[(3-chloro-4-fluorophenyl)amino]-7-[(3S)-tetrahydro-3-furanyloxy]-6-quinazolinyl]-4-(dimethylamino)-, (2E)-, (2Z)-2-butenedioate (1:2) is an FDA-approved EGFR inhibitor. Afatinib is typically administered as a dimaleate salt, and has the following structure:

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20 Afatinib

[0009] See U.S. Patent Nos. 6,251,912; 8,426,586; 8,545,884; and RE4343 1.

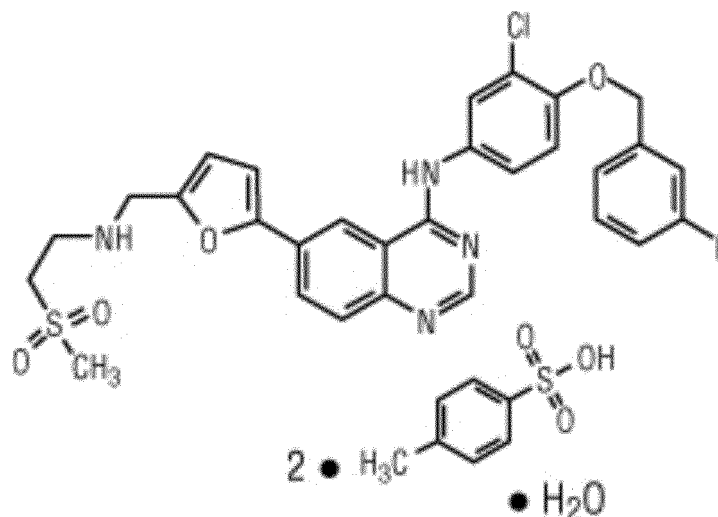
[0010] Lapatinib (*N*-(3-chloro-4-((3-fluorophenyl)methyl)oxy)phenyl)-6-[5-((2-(methylsulfonyl)ethyl)amino)methyl)-2-furanyl]-4-quinazolinaminebis(4-methylbenzenesulfonate) monohydrate) is an FDA-approved EGFR inhibitor. Lapatinib is typically administered as a ditosylate monohydrate with the following formula:

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Lapatinib

45 [0011] See International Patent Application PCT/EP99/00048, published as WO 99/35146 and PCT/US01/20706, published as WO 02/02552.

[0012] Cetuximab is an FDA-approved inhibitor of the EGFR receptor. Cetuximab is a chimeric (mouse/human) monoclonal antibody binds specifically to the extracellular domain of the human EGFR. Cetuximab is reported, for example, in United States Patent No. 6,217,866.

50 [0013] Despite advances in the treatment of HCC, there is a need to provide improved treatment for HCC.

SUMMARY

55 [0014] The present invention provides a combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof for use in a method of treating hepatocellular carcinoma in a patient in need thereof.

[0015] Moreover, the present invention provides a combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-

methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof for use in a method of reducing hepatocellular carcinoma cell viability or hepatocellular carcinoma cell proliferation by administering the combination to one or more cells.

[0016] In certain embodiments the EGFR inhibitor is gefitinib. In other embodiments the EGFR inhibitor is afatinib. In still other embodiments the EGFR inhibitor is lapatinib. In yet other embodiments the EGFR inhibitor is cetuximab. Use of the combination of Compound 1 and an EGRF inhibitor may lead to a synergistic reduction in the viability and/or proliferation of the cells. In certain embodiments the cells are hepatocellular cells. In certain embodiments the reduction in the viability and/or proliferation of the cells is an *in vitro* reduction.

[0017] Embodiments may provide a combination therapy, comprising an effective amount of Compound 1 and an effective amount of an EGFR inhibitor. In certain embodiments the EGFR inhibitor is gefitinib. In other embodiments the EGFR inhibitor is afatinib. In still other embodiments the EGFR inhibitor is lapatinib. Combination therapy provided herein may lead to a synergistic reduction in the viability of HCC cells and may lead to tumor growth inhibition of HCC in patients in need of treatment.

[0018] In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage between 50 mg to 600 mg. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage between 200 mg to 400 mg. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage of 300 mg.

[0019] In some embodiments the EGFR inhibitor is selected from, for example, 4-quinazolinamine *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy] (gefitinib), *N*-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[[(3*S*)-tetrahydro-3-furanyl]oxy]-6-quinazoliny]-4(dimethylamino)-2-butenamide) (afatinib), and *N*-(3-chloro-4-[[[(3-fluorophenyl)methyl]oxy]phenyl]-6-[5-[[2-(methylsulfonyl)ethyl]amino]methyl]-2-furanyl]-4-quinazolinamine bis(4-methylbenzenesulfonate) monohydrate (lapatinib).

[0020] In some embodiments the EGFR inhibitor is gefitinib. Gefitinib may be administered, for example in a daily dosage of 250 mg.

[0021] In some embodiments the EGFR inhibitor is afatinib. Afatinib may be administered, for example, in a daily dosage of 20 mg/day. Afatinib may be administered, for example, in a daily dosage of 30 mg/day. Afatinib may be administered, for example, in a daily dosage of 40 mg/day.

[0022] In some embodiments the EGFR inhibitor is lapatinib. Lapatinib may be administered, for example, in a daily dosage of between 1000 mg to 1500 mg. Lapatinib may be administered, for example, in a daily dosage of 1000 mg. Lapatinib may be administered, for example, in a daily dosage of 1250 mg. Lapatinib may be administered, for example, in a daily dosage of 1500 mg.

[0023] In some embodiments the EGFR inhibitor is cetuximab. Cetuximab is commercially available as ERBITUX® from Eli Lilly and Company and its affiliates, which provide it as 100 mg/50ml and 200 mg/100ml vials. Cetuximab is typically administered by injection for intravenous infusion. Dosage of cetuximab typically involves premedication with an H₁ antagonist, followed by administration of a 400 mg/m² initial dose as a 120-minute intravenous infusion followed by 250 mg/m² weekly infused over 60 minutes. In some embodiments the infusion rate is reduced by 50% if an infusion reaction should occur.

[0024] In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are administered as separate formulations in any order. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are administered as a single formulation. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are administered sequentially. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are administered simultaneously.

[0025] In some embodiments the form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide that is administered is the free base form. In some embodiments the form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide that is administered is a hydrochloride salt form.

[0026] The invention further provides a pharmaceutical formulation comprising *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof are administered sequentially.

nyl)acrylamide is a free base form. In some embodiments the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a hydrochloride salt form.

BRIEF DESCRIPTION OF THE DRAWINGS

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[0027]

FIG. 1A shows inhibition of cell viability as measured by CellTiter-Glo for Compound 1 and gefitinib.

FIG. 1B shows excess inhibition over Loewe additivity for Compound 1 and gefitinib.

10 FIG. 2A shows inhibition of cell viability as measured by CellTiter-Glo for Compound 1 and afatinib.

FIG. 2B shows excess inhibition over Loewe additivity for Compound 1 and afatinib.

FIG. 3A shows inhibition of cell viability as measured by CellTiter-Glo for Compound 1 and lapatinib.

FIG. 3B shows excess inhibition over Loewe additivity for Compound 1 and lapatinib.

FIG. 4A and FIG. 4B show Compound 1 and cetuximab synergistically inhibit growth of HEP3B cells *in vitro*.

15 FIG. 5A shows tumor volume for gefitinib as a single agent; FIG. 5B and FIG. 5C show tumor volume for combinations of gefitinib and Compound 1.

DETAILED DESCRIPTION OF EMBODIMENTS

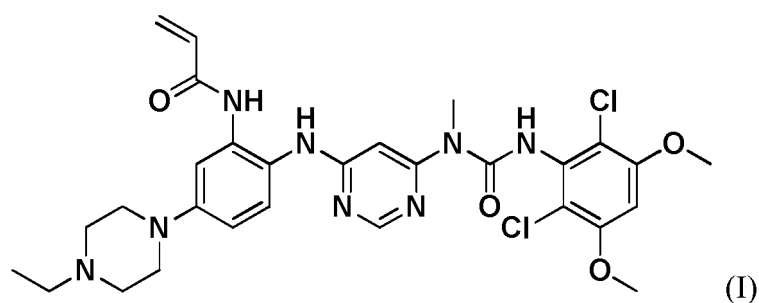
20 [0028] Provided herein are combinations of Compound 1 or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof for use in a method of treating hepatocellular carcinoma in a patient in need thereof. Further provided herein are combinations of Compound 1 or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof for use in a method of reducing hepatocellular carcinoma cell viability or hepatocellular carcinoma cell proliferation by administering the combination to one or more cells. In certain embodiments the EGFR inhibitor is gefitinib. In other embodiments, the EGFR inhibitor is afatinib. In still other embodiments, the EGFR inhibitor is lapatinib. In still other embodiments, the EGFR inhibitor is cetuximab. Such combinations may lead to a synergistic reduction in cell viability and/or cell proliferation. In certain embodiments the reduction in cell viability and/or cell proliferation is an *in vitro* reduction.

25 [0029] Provided herein are therapies useful in reducing the viability or proliferation of one or more cells. Such therapies may be useful for treating hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (IHCC). In some embodiments, the combination therapies include administration of Compound 1 in combination with an EGFR inhibitor. In certain embodiments the EGFR inhibitor is gefitinib. In other embodiments, the EGFR inhibitor is afatinib. In still other embodiments, the EGFR inhibitor is lapatinib.

30 [0030] As used herein, a "combination of therapeutic agents" and similar terms refer to a combination of two types of therapeutic agents: (1) Compound 1 and/or pharmacologically active salts thereof and (2) an EGFR inhibitor, and/or pharmacologically active salts thereof. "Combination" as used herein (including in the term "combination of therapeutic agents") refers to these types of therapeutic agents co-formulated in a single dosage form, individually formulated and co-administered, or individually formulated and sequentially administered.

35 [0031] Compound 1 is a selective, orally bioavailable small molecule FGFR4 inhibitor with the structure shown in Formula I:

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55 [0032] Compound 1 and its synthesis are reported in PCT International Application Publication No. WO2015/057938, published on April 23, 2015. Compound 1 may also be used alone or in combinations described herein as treatment for HCC or cholangiocarcinoma, including intrahepatic cholangiocarcinoma (IHCC). When used alone or in combinations as described herein, Compound 1 may be administered to patients in any of the following daily dosage amounts: 150 mg, 300 mg, 600 mg, 1000 mg, 1500 mg or 2000 mg. The daily dosage amount may be from 50 mg to 3000 mg, from 50 mg to 600 mg, or from 200 mg to 400 mg. The daily dosage may be part of a cyclic regimen lasting 14 days or 21

days. The daily dosage amount may be administered as a single dosage or as multiple dosages.

[0033] EGFR inhibitors suitable for use herein may include neutralizing monoclonal antibodies, including for example cetuximab, which is an immunoglobulin G1 human-murine chimeric counterpart of the murine monoclonal antibody M225; and panitumumab, which is a fully human IgG2 targeting the extracellular domains of EGFR monoclonal antibody, as reported in Martinelli, E, et al., 2009 "Anti-epidermal Growth Factor Receptor Monoclonal Antibodies in Cancer Therapy." Clin. Exper. Immuno. 158: 1-9. EGFR inhibitors may further include tyrosine kinase inhibitors, including for example gefitinib, erlotinib, lapatinib, icotinib, KD019, varlitinib, BMS599626, JNJ26483327, TAK285, AL6802, PKI166, AEE788, afatinib, dacomitinib, neratinib, poziotinib, BMS690514, CUDC101, KKI357, AV412, canertinib, pelitinib, WZ4002, CO-1686, AZD-9291, HM61713, and TAS-2913. See, for example, Lee, C, et al., 2014 "Small-molecule EGFR Tyrosine Kinase Inhibitors for the Treatment of Cancer." Expert Opin. Investig. Drugs 23(10): 1333-1348.

[0034] Administration of a combination of therapeutic agents may comprise administration of the individual therapeutic agents in combination in a single formulation or unit dosage form, administration of the individual therapeutic agents of the combination concurrently but separately, or administration of the individual agents of the combination sequentially by any suitable route. The dosage of the individual therapeutic agents of the combination may require more frequent administration of one of the agents as compared to the other agent in the combination. Therefore, to permit appropriate dosing, packaged pharmaceutical products may contain one or more dosage forms that contain the combination of agents, and one or more dosage forms that contain one of the combinations of agents, but not the other agent(s) of the combination.

[0035] Combinations as reported herein may include embodiments wherein one or more of Compound 1 and an EGFR inhibitor are administered as a pharmaceutically acceptable salt or as a free base. There is no requirement that both compounds be administered as the same pharmaceutically acceptable salt, but they may be. In particular embodiments combinations comprise a free base form of Compound 1 and an EGFR inhibitor. In other embodiments combinations comprise an HCl form of Compound 1 and an EGFR inhibitor.

[0036] "Pharmaceutically acceptable salt" as used herein refers to acid addition salts or base addition salts of the compounds in the present disclosure. A pharmaceutically acceptable salt is any salt which retains the activity of the parent compound and does not impart any unduly deleterious or undesirable effect on a subject to whom it is administered and in the context in which it is administered. Pharmaceutically acceptable salts include, but are not limited to, metal complexes and salts of both inorganic and carboxylic acids. Pharmaceutically acceptable salts also include metal salts such as aluminum, calcium, iron, magnesium, manganese and complex salts. In addition, pharmaceutically acceptable salts include, but are not limited to, acid salts such as acetic, aspartic, alkylsulfonic, arylsulfonic, axetil, benzenesulfonic, benzoic, bicarbonic, bisulfuric, bitartaric, butyric, calcium edetate, camrylic, carbonic, chlorobenzoic, citric, edetic, edisyllic, estolic, esyl, esylic, formic, fumaric, gluceptic, gluconic, glutamic, glycolic, glycolylarsanilic, hexamic, hexylresorcinoic, hydrabamic, hydrobromic, hydrochloric, hydroiodic, hydroxynaphthoic, isethionic, lactic, lactobionic, maleic, malic, malonic, mandelic, methanesulfonic, methylnitric, methylsulfuric, mucic, muconic, napsylic, nitric, oxalic, p-nitromethanesulfonic, pamoic, pantothenic, phosphoric, monohydrogen phosphoric, dihydrogen phosphoric, phthalic, polygalactouronic, propionic, salicylic, stearic, succinic, sulfamic, sulfanilic, sulfonic, sulfuric, tannic, tartaric, teodic, toluenesulfonic, and the like.

[0037] Embodiments may be hydrochloride salts. Pharmaceutically acceptable salts may be derived from amino acids including, but not limited to, cysteine. Methods for producing compounds as salts are known to those of skill in the art (see, e.g., Stahl et al., Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wiley-VCH; Verlag Helvetica Chimica Acta, Zurich, 2002; Berge et al., J. Pharm. Sci. 66: 1, 1977).

[0038] An "effective amount" of a combination of therapeutic agents (e.g., Compound 1 and an EGFR inhibitor) is an amount sufficient to provide an observable therapeutic benefit compared to HCC or IHCC left untreated in a subject or patient.

[0039] Active agents as reported herein can be combined with a pharmaceutically acceptable carrier to provide pharmaceutical formulations thereof. The particular choice of carrier and formulation will depend upon the particular route of administration for which the composition is intended.

[0040] "Pharmaceutically acceptable carrier" as used herein refers to a nontoxic carrier, adjuvant, or vehicle that does not destroy the pharmacological activity of the compound with which it is formulated. Pharmaceutically acceptable carriers, adjuvants or vehicles that may be used in the compositions of this invention include, but are not limited to, sorbic acid, potassium sorbate, partial glyceride mixtures of saturated vegetable fatty acids, water, salts or electrolytes, disodium hydrogen phosphate, potassium hydrogen phosphate, sodium chloride, zinc salts, colloidal silica, magnesium trisilicate, polyvinyl pyrrolidone, cellulose-based substances, polyethylene glycol, sodium carboxymethylcellulose, polyacrylates, waxes, polyethylene glycol and wool fat.

[0041] The compositions of the present invention may be suitable for parenteral, oral, inhalation spray, topical, rectal, nasal, buccal, vaginal or implanted reservoir administration, etc. In some embodiments, the formulation comprises ingredients that are from natural or non-natural sources. In some embodiments, the formulation or carrier may be provided in a sterile form. Non-limiting examples of a sterile carrier include endotoxin-free water or pyrogen-free water.

[0042] The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In particular embodiments, the compounds are administered intravenously, orally, subcutaneously, or via intramuscular administration. Sterile injectable forms of the compositions of this invention may be aqueous or oleaginous suspension. These suspensions may be formulated according to techniques known in the art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a sterile injectable solution or suspension in a nontoxic parenterally acceptable diluent or solvent. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium.

[0043] For this purpose, any bland fixed oil may be employed including synthetic mono- or diglycerides. Fatty acids and their glyceride derivatives are useful in the preparation of injectables, as are natural pharmaceutically acceptable oils, such as olive oil or castor oil, especially in their polyoxyethylated versions. These oil solutions or suspensions may also contain a long-chain alcohol diluent or dispersant, such as carboxymethyl cellulose or similar dispersing agents that are commonly used in the formulation of pharmaceutically acceptable dosage forms including emulsions and suspensions. Other commonly used surfactants, such as Tweens, Spans and other emulsifying agents that are commonly used in the manufacture of pharmaceutically acceptable solid, liquid, or other dosage forms may also be used for the purposes of formulation.

[0044] For oral administration, a compound or salt may be provided in an acceptable oral dosage form, including, but not limited to, capsules, tablets, aqueous suspensions or solutions. In the case of tablets for oral use, carriers commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, may also be added. For oral administration in a capsule form, useful diluents include lactose and dried cornstarch. When aqueous suspensions are required for oral use, the active ingredient may be combined with emulsifying and suspending agents. If desired, certain sweetening, flavoring or coloring agents may also be added. In addition preservatives may also be added. Suitable examples of pharmaceutically acceptable preservatives include, but are not limited to, various antibacterial and antifungal agents such as solvents, for example ethanol, propylene glycol, benzyl alcohol, chlorobutanol, quaternary ammonium salts, and parabens (such as methyl paraben, ethyl paraben, propyl paraben, etc.).

[0045] "Immediate-release" is meant to include a conventional release, in which release of the drug starts immediately after administration. As used herein, the term "immediate release" includes dosage forms that allow the drug to dissolve in the gastrointestinal contents, with no intention of delaying or prolonging the dissolution or absorption of the drug. The objective is for the drug to be released rapidly after administration, for example for it to be possible to release at least 80% of the drug within approximately 30 minutes after commencement of dissolution in a dissolution test.

[0046] "Sustained-release" or "extended-release" includes dosage forms whose drug-release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as a solution or an immediate release dosage form.

[0047] The term "steady-state" means that a plasma level for a given active agent or combination of active agents, has been achieved and which is maintained with subsequent doses of the active agent(s) at a level which is at or above the minimum effective therapeutic level and is below the minimum toxic plasma level for a given active agent(s).

[0048] The term "single formulation" as used herein refers to a single carrier or vehicle formulated to deliver effective amounts of both therapeutic agents to a patient. The single vehicle is designed to deliver an effective amount of each of the agents along with any pharmaceutically acceptable carriers or excipients. In some embodiments, the vehicle is a tablet, capsule, pill, or a patch.

[0049] The term "unit dose" is used herein to mean simultaneous administration of both agents together, in one dosage form, to the patient being treated. In some embodiments, the unit dose is a single formulation. In certain embodiments, the unit dose includes one or more vehicles such that each vehicle includes an effective amount of at least one of the agents (Compound 1 or an EGFR inhibitor) along with pharmaceutically acceptable carriers and excipients. In some embodiments, the unit dose is one or more tablets, capsules, pills, or patches administered to the patient at the same time.

[0050] The term "dose range" as used herein refers to an upper and a lower limit of an acceptable variation of the amount of agent specified. Typically, a dose of an agent in any amount within the specified range can be administered to patients undergoing treatment.

[0051] The term "treat" is used herein to mean to relieve, reduce or alleviate at least one symptom of a disease in a subject. For example, in relation to HCC, the term "treat" may mean to arrest, delay the onset (i.e., the period prior to clinical manifestation of a disease or symptom of a disease) and/or reduce the risk of developing or worsening a symptom of a disease. The term "protect" is used herein to mean prevent delay or treat, or all, as appropriate, development or continuance or aggravation of symptoms of the disease in a subject.

[0052] The term "subject" or "patient" is intended to include animals, which are capable of suffering from or afflicted with HCC or IHCC. Examples of subjects or patients include mammals, e.g., humans, dogs, cows, horses, pigs, sheep, goats, cats, mice, rabbits, rats, and transgenic non-human animals. In certain embodiments, the subject is a human, e.g., a human suffering from, at risk of suffering from, or potentially capable of suffering from HCC or IHCC.

[0053] The term "about" or "approximately" usually means within 20%, more preferably within 10%, and most preferably still within 5% of a given value or range. Alternatively, especially in biological systems, the term "about" means within about a log (i.e., an order of magnitude) preferably within a factor of two of a given value.

[0054] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein.

[0055] The term "synergistic effect" as used herein, refers to action of two agents such as, for example, Compound 1 and gefitinib, producing an effect, for example, reducing cell viability and/or cell proliferation or slowing the progression of HCC, which is greater than the simple addition of the effects of each drug administered by themselves. A synergistic effect can be calculated, for example, using suitable methods such as the Sigmoid-Emax equation (Holford, N. H. G. and Scheiner, L. B., Clin. Pharmacokinet. 6: 429-453 (1981)), the equation of Loewe additivity (Loewe, S. and Muischnek, H., Arch. Exp. Pathol Pharmacol. 114: 313-326 (1926)) and the median-effect equation (Chou, T. C. and Talalay, P., Adv. Enzyme Regul. 22: 27-55 (1984)). Each equation referred to above can be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

[0056] In some embodiments, treatment is provided to a subject having hepatocellular carcinoma with altered FGFR4 and/or FGF19 (fibroblast growth factor 19) status.

[0057] In some embodiments, treatment may include or be performed in conjunction with analyzing FGFR4 and/or FGF19 status in a biological sample containing cells of said hepatocellular carcinoma, and if said hepatocellular carcinoma exhibits an FGFR4 and/or FGF19 alteration, treating a subject with a treatment effective amount of a therapeutic combination as described herein.

Medical uses

[0058] Provided herein is a combination therapy for use in a method of treating hepatocellular carcinoma in a patient in need thereof. Further provided herein is a combination therapy for use in a method of reducing hepatocellular carcinoma cell viability or hepatocellular carcinoma cell proliferation by administering the combination to one or more cells. Such a combination may be useful for the treatment of HCC or IHCC. As discussed below, combinations provided herein may have a number of advantages.

[0059] One advantage of the combination disclosed herein is the unexpected synergistic effect of a combination of Compound 1 and an EGFR inhibitor on: (1) the reduction in the viability and/or proliferation of one or more cells; and (2) tumor growth inhibition and treatment of HCC or IHCC.

[0060] In some embodiments, provided herein is a single pharmaceutical formulation containing a combination of Compound 1 and an EGFR inhibitor. An advantage provided herein is the synergistic effect that results in the reduction of the viability and/or proliferation of a cell or in the treatment of HCC compared to treatment with a single dose of either drug. When the drugs are provided in a single unit dose or single formulation, the "pill burden" on a patient suffering from HCC is not increased.

[0061] As specified above, in one aspect, provided herein is a drug combination useful for treating, preventing, arresting, delaying the onset of and/or reducing the risk of developing, or reversing HCC in a mammal comprising administering to said mammal a combination therapy, comprising an effective amount of Compound 1 and an effective amount of an EGFR inhibitor..

[0062] In some embodiments, the subject to be treated (e.g., patient) is determined to be non-responsive or resistant to one or more HCC therapies, e.g., Compound 1. In other embodiments, the individual to be treated is responsive to Compound 1 therapy, but the therapy was improved with the administration of an EGFR inhibitor. For example, the patient is administered Compound 1 (e.g., 50 mg to 600 mg per day, 200 mg to 400 mg per day, or 300 mg per day for some period of time, e.g., more than one day, more than two days, more than three days, more than one week, more than one month, etc. After that time, an EGFR inhibitor could be administered to that patient in combination with Compound 1.

[0063] Amounts of EGFR inhibitor may vary depending on the EGFR inhibitor that is used. For example, gefitinib may be administered in an amount of 250 mg/day or 500 mg/day; afatinib may be administered at 10 -50 mg/day, at 20-40 mg/day, at 20 mg/day, at 30 mg/day, or at 40 mg/day; and lapatinib may be administered at 100 mg/day to 1500 mg/day, at 1250 mg/day to 4500 mg/day, at 1500 mg/day to 5500 mg/day, at 500 mg/day, at 750 mg/day, at 1000 mg/day, at 1250 mg/day, and at 1500 mg/day. The daily dosage may be part of a cyclic regimen lasting 14 to 21 days or longer. The daily dosage amount may be administered as a single dosage or as multiple dosages.

[0064] One skilled in the art appreciates that the effective dose of the active drug may be lower than the actual amount administered. As such, provided herein are doses necessary to achieve a therapeutic dose.

[0065] In various embodiments, provided herein are combinations for use in a method of treating HCC by administering an effective amount of Compound 1 and an EGFR inhibitor, to an individual having HCC. The amount of the combination of agents may be effective to treat the HCC. In one embodiment, the combination of agents has a synergistic effect. In one embodiment, even though one or more of the agents administered alone at a particular dosage may be effective, when administered in combination, at the same dosage of each agent, the treatment is more effective. For example, in one embodiment a combination of Compound 1 and gefitinib is more effective than is administration of either agent alone. In another embodiment a combination of Compound 1 and afatinib is more effective than is administration of either agent alone. In another embodiment a combination of Compound 1 and lapatinib is more effective than is administration of either agent alone.

Dosages

[0066] The optimal dose of the combination of agents for treatment of HCC can be determined empirically for each individual using known methods and will depend upon a variety of factors, including the activity of the agents; the age, body weight, general health, gender and diet of the individual; the time and route of administration; and other medications the individual is taking. Optimal dosages may be established using routine testing and procedures that are well known in the art.

[0067] For the combination therapy of the instant invention, the daily dose of Compound 1 is in the range of 50 mg to 600 mg. In some embodiments, the daily dose of Compound 1 is up to 600 mg. In certain embodiments, the daily dose of Compound 1 is up to 400 mg. In various embodiments, the daily dose of Compound 1 is up to 300 mg. In certain embodiments, the daily dose of Compound 1 is 200 mg to 400 mg. In one embodiment, the daily dose is 300 mg.

[0068] The time of administration can be chosen such that both the drugs are administered simultaneously, separately or sequentially, either in the morning or at night. Alternatively, one drug can be administered in the morning and the other at night. In certain embodiments, both the drugs can be administered as a single tablet, capsule, pill, patch or jelly formulation, once daily, either in the morning or at night.

[0069] The amount of combination of agents that may be combined with the carrier materials to produce a single dosage form will vary depending upon the individual treated and the particular mode of administration. In some embodiments the unit dosage forms containing the combination of agents as described herein will contain the amounts of each agent of the combination that are typically administered when the agents are administered alone.

Pharmaceutical Formulations and Routes of Administration

[0070] Provided herein are pharmaceutical formulations comprising *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof. Such formulations may be useful for the treatment of HCC. The pharmaceutical formulations may additionally comprise a carrier or excipient, stabilizer, flavoring agent, and/or coloring agent.

[0071] A combination of agents may be administered using a variety of routes of administration known to those skilled in the art. Routes of administration include oral administration. In certain embodiments, a pharmaceutical formulation comprising a combination of agents may be taken orally in the form of liquid, syrup, tablet, capsule, powder, sprinkle, chewtab, or dissolvable disk. Alternatively, pharmaceutical formulations of the present invention can be administered intravenously or transdermally. Additional routes of administration are known to those skilled in the art (see, e.g., Remington's Pharmaceutical Sciences, Gennaro A. R., Ed., 20. sup.th Edition, Mack Publishing Co., Easton, Pa.).

[0072] In some embodiments, the Compound 1 and EGFR inhibitor are formulated as a paste, jelly, or suspension. For example, the drugs are dissolved, entrapped or suspended in the form of drug particles, microencapsulated particles, or drug-polymer particles in a gelatinous solution or semi-solid. An advantage of an oral jelly formulation is that it is easier to administer the drugs to patients who have difficulty swallowing tablets, capsules or pills. In certain embodiments, both agents are thoroughly mixed and suspended in an appropriate medium to form a paste or a gel. Additional agents can optionally be mixed to provide flavor during oral administration. Peanut butter or alginate, flavored with raspberry and a sweetener are examples of the many suitable taste masking agents. In various embodiments, the paste or jelly can also be formulated with suitable binders or excipients known in the art for topical administration.

[0073] Methods of preparing sustained release formulations in the form of tablets, capsules or pills are known in the art. In some embodiments, the sustained release formulation is prepared by coating the active ingredient of the drug with a polymer, preferably a water-insoluble polymer. For example, a water-insoluble polymer used in the pharmaceutical field as a sustained release coating agent, an enteric coating agent, or a gastric coating agent. The water-insoluble polymer can include, for example, ethyl cellulose, purified shellac, white shellac, aminoalkyl methacrylate copolymer

RS, hydroxypropyl methylcellulose phthalate, hydroxypropyl methylcellulose acetate succinate, carboxymethylcellulose, cellulose acetate phthalate, methacrylic acid copolymer L, methacrylic acid copolymer LD, methacrylic acid copolymer S, aminoalkyl methacrylate copolymer E, or polyvinyl acetal diethylaminoacetate.

[0074] The type, degree of substitution and molecular weight of the water-insoluble polymers can depend on solubility of the active ingredient in water or an alcohol, the desired sustained release level and the like. The water-insoluble polymers can be used either alone or in combination. There can be further incorporated a hydrogenated oil, stearic acid, or cetanol as a coating auxiliary agent, and a middle-chain triglyceride, triacetin, triethyl citrate, or cetanol as a plasticizer.

[0075] In some embodiments, the sustained release formulation is a matrix-type tablet or granule. The active ingredient can be coated with up to 3 different types of polymers. These three different types of polymers can include: 1) a water insoluble polymer, such as ethylcellulose; 2) a pH independent gelling polymer, such as hydroxypropyl methylcellulose; and 3) a pH dependent gelling polymer, such as sodium alginate. These three different types of polymers can be used together to attenuate the release rate of the drugs.

Dosage Forms: Release Properties

[0076] Sustained-release formulations can achieve a degree of sustained effect. However, the exposure and/or the bioavailability of the active ingredient may vary based on a variety of factors, such as for example, the absorption window, the carriers or excipients used in the formulation, the mode of delivery of the formulation, and/or the transit time of the active ingredient through the gastrointestinal tract of the patient.

[0077] A combination therapy can contain at least one sustained-release portion for performing a sustained-release function and one immediate release portion for performing an immediate release function. In certain embodiments, when the combination therapy is in a single dosage form, it can be in the form of tablets formed from a mixture of sustained-release granules constituting a sustained-release portion and immediate-release granules constituting an immediate-release portion, a capsule preparation obtained by filling a capsule with sustained-release granules and immediate-release granules, or press-coated tablets in which an outer layer constituting an immediate-release portion is formed on an inner core constituting a sustained-release portion. There is, however, no limitation to the above embodiments.

[0078] Moreover, there are no particular limitations on the state of containment of each drug in the composition or in an immediate-release portion or a sustained-release portion; the Compound 1 may be dispersed uniformly in the composition, immediate release portion or sustained release portion, or may be contained in only one part of the composition, immediate-release portion or sustained-release portion, or may be contained such that there is a concentration gradient.

[0079] A sustained-release portion in the composition according to the present invention can contain at least one non-pH-dependent polymeric substance or pH-dependent polymeric substance for controlling drug release.

[0080] A non-pH-dependent polymeric substance used herein can comprise a polymeric substance whose charge state hardly changes under pH conditions generally found in the gastrointestinal tract, specifically from pH 1 to pH 8. This means, for example, a polymeric substance that does not have functional groups whose charge state changes depending on the pH such as basic functional groups such as amino groups or acidic functional groups such as carboxylic acid groups. Note that the non-pH-dependent polymeric substance can be included for giving the composition according to the present invention a sustained-release function, but may also be included for another purpose. Moreover, the non-pH-dependent polymeric substance used in the present invention may be water-insoluble, or may swell in water or dissolve in water to form a gel.

[0081] Examples of water-insoluble non-pH-dependent polymeric substances include, but are not limited to, cellulose ethers, cellulose esters, and methacrylic acid-acrylic acid copolymers (trade name Eudragit, manufactured by Rohm GmbH & Co. KG, Darmstadt, Germany). Examples include, but are not limited to, cellulose alkyl ethers such as ethylcellulose (trade name Ethocel, manufactured by Dow Chemical Company, USA), ethyl methylcellulose, ethyl propylcellulose or isopropylcellulose, and butylcellulose, cellulose aralkyl ethers such as benzyl cellulose, cellulose cyanoalkyl ethers such as cyanoethylcellulose, cellulose organic acid esters such as cellulose acetate butyrate, cellulose acetate, cellulose propionate or cellulose butyrate, and cellulose acetate propionate, ethyl acrylate-methyl methacrylate copolymers (trade name Eudragit NE, manufactured by Rohm GmbH & Co. KG, Darmstadt, Germany), and aminoalkyl methacrylate copolymer RS (trade names Eudragit RL, Eudragit RS). There are no particular limitations on the mean particle diameter of a water-insoluble polymer used in the present invention, but usually the lower this mean particle diameter the better the performance, with the mean particle diameter preferably being from 0.1 to 100 μm , more preferably from 1 to 50 μm , particularly preferably from 3 to 15 μm , most preferably from 5 to 15 μm . Moreover, examples of water-soluble or water-swelling non-pH-dependent polymeric substances include, but are not limited to, polyethylene oxide (trade name Polyox, manufactured by Dow Chemical Company, molecular weight 100,000 to 7,000,000), low-substituted hydroxypropyl cellulose (trade name L-HPC, manufactured by Shin-Etsu Chemical, Japan), hydroxypropyl cellulose (trade name HPC, manufactured by Nippon Soda, Co., Ltd, Japan), hydroxypropyl methylcellulose (trade names Metolose 60SH, 65SH, 90SH, manufactured by Shin-Etsu Chemical, Japan), and methylcellulose (trade name Metolose SM, manufactured by Shin-Etsu Chemical, Japan).

[0082] In some embodiments a single non-pH-dependent polymeric substance may be contained in the composition, or a plurality of the non-pH-dependent polymeric substances may be contained. The non-pH-dependent polymeric substance, if used in embodiments reported herein, may be a water-insoluble polymeric substance, more preferably ethylcellulose, an ethyl acrylate-methyl methacrylate copolymer (trade name Eudragit NE), or an aminoalkyl methacrylate copolymer RS (trade name Eudragit RL, Eudragit RS). Particularly preferable is at least one of ethylcellulose and an aminoalkyl methacrylate copolymer RS. Most preferable is ethylcellulose. There are no particular limitations on the amount of the non-pH-dependent polymeric substance contained in the composition; this amount can be adjusted as appropriate in accordance with the purpose such as controlling sustained drug release.

[0083] A pH-dependent polymeric substance that can be used in embodiments reported herein may be a polymeric substance whose charge state changes under pH conditions generally found in the gastrointestinal tract, specifically from pH 1 to pH 8. This means, for example, a polymeric substance having functional groups whose charge state changes depending on the pH such as basic functional groups such as amino groups or acidic functional groups such as carboxylic acid groups. The pH-dependent functional groups of the pH-dependent polymeric substance are preferably acidic functional groups, with the pH-dependent polymeric substance most preferably having carboxylic acid groups.

[0084] A pH-dependent polymeric substance used in the present invention may be water-insoluble, or may swell in water or dissolve in water to form a gel. Examples of pH-dependent polymeric substances used in the present invention include, but are not limited to, enteric polymeric substances. Examples of enteric polymeric substances include, but are not limited to, methacrylic acid-methyl methacrylate copolymers (Eudragit L100, Eudragit S100, manufactured by Rohm GmbH & Co. KG, Darmstadt, Germany), methacrylic acid-ethyl acrylate copolymers (Eudragit L100-55, Eudragit L30D-55, manufactured by Rohm GmbH & Co. KG, Darmstadt, Germany), hydroxypropyl methylcellulose phthalate (HP-55, HP-50, manufactured by Shin-Etsu Chemical, Japan), hydroxypropyl methylcellulose acetate succinate (AQOAT, manufactured by Shin-Etsu Chemical, Japan), carboxymethyl ethylcellulose (CMEC, manufactured by Freund Corporation, Japan), and cellulose acetate phthalate.

[0085] Examples of pH-dependent polymeric substances that swell in water or dissolve in water to form a gel include, but are not limited to, alginic acid, pectin, carboxyvinyl polymer, and carboxymethyl cellulose. In the present invention, a single pH-dependent polymeric substance may be contained in the composition, or a plurality of pH-dependent polymeric substances may be contained. The pH-dependent polymeric substance used in the present invention is preferably an enteric polymeric substance, more preferably a methacrylic acid-ethyl acrylate copolymer, a methacrylic acid-methyl methacrylate copolymer, hydroxypropyl methylcellulose phthalate, or hydroxypropyl methylcellulose acetate succinate, particularly preferably a methacrylic acid-ethyl acrylate copolymer.

[0086] When using a pH-dependent polymeric substance in the manufacturing process of a composition according to the present invention, a commercially available product of a powder type or a granular type, or a suspension type in which the pH-dependent polymeric substance has been dispersed in a solvent in advance can be used as is, or such a commercially available product can be used dispersed in water or an organic solvent. The lower the particle diameter of the pH-dependent polymeric substance the better the performance, with the pH-dependent polymeric substance preferably being of the powder type. In the case of a methacrylic acid-ethyl acrylate copolymer, an example is Eudragit L100-55. There are no particular limitations on the mean particle diameter of a pH-dependent polymeric substance used in the present invention, but the mean particle diameter is preferably from 0.05 to 100 μm , more preferably from 0.05 to 70 μm , most preferably from 0.05 to 50 μm . Moreover, there are no particular limitations on the amount of the pH-dependent polymeric substance, for example, in the case of an enteric polymeric substance, the amount is generally from 0.1 to 90 parts by weight, preferably from 1 to 70 parts by weight, more preferably from 5 to 60 parts by weight, particularly preferably from 10 to 50 parts by weight, based on 100 parts by weight of the composition.

[0087] A combination therapy according to embodiments reported herein may further contain any of various additives, such as any of various pharmacologically acceptable carriers such as diluents, lubricants, binders and disintegrants, as well as preservatives, colorants, sweeteners, plasticizers, film coating agents and so on, as necessary. Examples of diluents include, but are not limited to, lactose, mannitol, dibasic calcium phosphate, starch, pregelatinized starch, crystalline cellulose, light silicic anhydride, synthetic aluminum silicate, magnesium aluminatemetasilicate or the like. Examples of lubricants include, but are not limited to, magnesium stearate, calcium stearate, talc, sodium stearyl fumarate or the like. Examples of binders include, but are not limited to, hydroxypropyl cellulose, methylcellulose, sodium carboxymethyl cellulose, hydroxypropyl methylcellulose, polyvinylpyrrolidone or the like. Examples of disintegrants include, but are not limited to, carboxymethyl cellulose, calcium carboxymethyl cellulose, croscarmellose sodium, sodium carboxymethyl starch, low-substituted hydroxypropyl cellulose or the like. Examples of preservatives include, but are not limited to, paraoxybenzoic acid esters, chlorobutanol, benzyl alcohol, phenethyl alcohol, dehydroacetic acid, sorbic acid or the like. Preferable examples of colorants include, but are not limited to, water-insoluble lake pigments, natural pigments (e.g., β -carotene, chlorophyll, red ferric oxide), yellow ferric oxide, red ferric oxide, black ferric oxide or the like. Preferable examples of sweeteners include, but are not limited to, sodium saccharin, dipotassium glycyrrhizate, aspartame, stevia or the like. Examples of plasticizers include, but are not limited to, glycerol fatty acid esters, triethyl citrate, propylene glycol, polyethylene glycol or the like. Examples of film coating agents include, but are not limited to,

hydroxypropyl methylcellulose, hydroxypropyl cellulose or the like.

Manufacturing Methods

5 **[0088]** To manufacture embodiments as reported herein, a single conventional method, or a combination of conventional methods, can be used. For example, when manufacturing drug-containing granules as a sustained-release portion or an immediate-release portion, granulation is the main operation, but this may be combined with other operations such as mixing, drying, sieving, and classification. As the granulation method, for example, a wet granulation method in which a binder and a solvent are added to the powder and granulation is carried out, a dry granulation method in which the powder is compressed and granulation is carried out, a molten granulation method in which a binder that melts on heating is added and heating and granulation are carried out, or the like can be used.

10 **[0089]** Furthermore, in accordance with the granulation method, an operating method such as a mixing granulation method using a planetary mixer, a screw mixer or the like, a high-speed mixing granulation method using a Henschel mixer, a Super mixer or the like, an extruding granulation method using a cylindrical granulator, a rotary granulator, a screw extruding granulator, a pellet mill type granulator or the like, a wet high-shear granulation method, a fluidized-bed granulation method, a compression granulation method, a crushing granulation method, or a spraying granulation method can be used. After the granulation, drying using a dryer, a fluidized bed or the like, cracking, and sieving can be carried out to obtain the granules or fine granules for use. Moreover, a granulation solvent may be used when preparing the composition according to the present invention. There are no particular limitations on such a granulation solvent, which may be water or any of various organic solvents, for example, water, a lower alcohol such as methanol or ethanol, a ketone such as acetone or methyl ethyl ketone, methylene chloride, or a mixture thereof.

15 **[0090]** For sustained-release granules contained in embodiments, at least one drug and at least one selected from non-pH-dependent polymeric substances and pH-dependent polymeric substances are mixed together, a diluent and a binder are added as necessary, and granulation is carried out to obtain granular matter. The granular matter obtained is dried using a tray dryer, a fluidized bed dryer or the like, and sieving is carried out using a mill or an oscillator, whereby the sustained-release granules can be obtained. Alternatively, as a method of manufacturing sustained-release granules in the present invention, it is possible to add at least one drug, at least one selected from non-pH-dependent polymeric substances and pH-dependent polymeric substances, and as necessary a diluent and a binder using a dry compactor such as a roller compactor or a slug tableting machine, and carry out compression-molding while mixing, and then carry out granulation by cracking down to a suitable size. The granular matter prepared using such a granulator may be used as is as granules or fine granules according to the present invention, or may be further cracked using a power mill, a roll granulator, a rotor speed mill or the like, and sieved to obtain sustained-release granules. Note that immediate-release granules can also be manufactured as for the sustained-release granules.

25 **[0091]** A compression-molded product can be manufactured as a drug-containing sustained-release portion or immediate-release portion, or as a composition reported herein using a single conventional method, or a combination of conventional methods. For example, at least one drug, at least one selected from non-pH-dependent polymeric substances and pH-dependent polymeric substances, a diluent such as mannitol or lactose, a binder such as polyvinylpyrrolidone or crystalline cellulose, a disintegrant such as carmellose sodium or crospovidone, and a lubricant such as magnesium stearate or talc are used, and tableting is carried out using an ordinary method, whereby the compression-molded product can be obtained. In this case, tableting is the main operation in the method of manufacturing the compression-molded product, but this may be combined with other operations such as mixing, drying, sugar coating formation, and coating.

30 **[0092]** Examples of the method for the tableting include, but are not limited to, direct compression molding in which at least one drug and pharmacologically acceptable additives are mixed together and then the mixture is directly compression-molded into tablets using a tableting machine, and dry granule compression or wet granule compression in which sustained-release granules or immediate-release granules according to the present invention are subjected to compression-molding after adding a lubricant or a disintegrant as necessary. There are no particular limitations on the tableting machine used in the compression molding; for example, a single-punch tableting machine, a rotary tableting machine, or a press-coated tableting machine can be used.

35 **[0093]** Drug-containing sustained-release granules or immediate-release granules, or compression-molded product according to embodiments herein can be used as is in the form of granules or a tablet as the composition, but may also be subjected to further processing to manufacture the composition. For example, the compression-molded product or granules can be given a film coating using a film base material such as ethylcellulose, casein, methylcellulose, hydroxypropyl methylcellulose, methacrylic acid copolymer L, cellulose acetate phthalate, shellac or the like, or given a sugar coating using a sugar coating liquid containing saccharose, sugar alcohol, gum arabic powder, talc or the like, thus producing film-coated tablets or sugar-coated tablets. One solvent in this coating technique may be purified water, but an organic solvent such as an alcohol, a ketone, an ether or a chlorinated hydrocarbon, or a mixture thereof can also be used. For example, ethanol, acetone, methylene chloride or the like can be used as an organic solvent. Moreover,

as the coating apparatus, an apparatus ordinarily used in coating techniques for manufacturing medicines can be used, with examples including a spray coating apparatus in which the coating is carried out by spraying a coating liquid or the like, and a rotor fluidized bed granulator for layering.

[0094] In the case of manufacturing capsule preparations, capsule preparations can be manufactured by filling sustained-release granules or immediate-release granules as above, or mini-tablets into hard gelatin capsules or HPMC capsules using an automatic capsule filling machine. Alternatively, in the case of the preparations for per-tube administration or a dry syrup that is used mixed with water or the like when taken, sustained-release granules or immediate-release granules as above can be mixed with a thickener or a dispersant so as to disperse these granules, the mixture then being made into granules or tablets. Furthermore, a liquid or jelly can be made using water, and substances selected from dispersants, emulsifiers, thickeners, preservatives, pH adjustors, sweeteners, flavorings, fragrances and so on. However, with respect to other manufacturing methods, there are no limitations to the above.

[0095] In order that embodiments described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting.

EXAMPLES

Materials and methods

Compounds and Drugs

[0096] Compound 1 is a selective, orally bioavailable small molecule FGFR4 inhibitor that is being developed by H3 Biomedicine. Gefitinib, afatinib, lapatinib, and cetuximab are FDA-approved EGFR inhibitors.

Cell Lines Tested

[0097] The cell line used, Hep3B (also called HEP3B.1-7), was sourced from American Type Culture Collection (ATCC), verified free of mycobacterium contamination and verified for identity by short tandem repeat analysis of 9 markers.

Cell Line Maintenance and Study Conditions

[0098] Medium conditions for growth included Eagle's Minimum Essential Medium (EMEM) (ATCC® 30-2003™) supplemented with 10% Fetal Bovine Serum (FBS) or RPMI1640 medium containing 10% FBS. Cells were maintained prior to and during experiments at 37 °C, 5% CO₂, and at 95% relative humidity. Cell passage number was limited to between 12 and 20. During in vitro experiments cells were seeded at appropriate densities to provide logarithmic growth during and at least 24 hours beyond the experiment target compound exposure duration.

Compound Preparation and Presentation to Cells

[0099] Compounds for assay were serially diluted in dimethyl sulfoxide (DMSO) using a low-volume liquid handler (VIAFLO ASSIST and VIAFLO II electronic 16-channel pipette, 0.5 - 12.5 μL) in an 11-point half-log serial dilution to create a master dose response source (MDR) used for all tests. Antibody solutions were serially diluted in phosphate buffered saline (PBS).

[0100] Transfers of compounds and antibodies from the MDR source plates to cell ARP's were accomplished directly by low-energy acoustic transfer (ATS100, EDC) using combination-specific transfer maps (TransferTrack, BioSero). After transfer of compounds and antibodies to the assay plate, the dose-response range experienced by cells was typically 5 μM - 50 pM and 30 nM - 0.5 pM (5 logs), respectively. The final DMSO concentration in the assay was 0.1% for compound-compound combination, uniformly, and 0.05% for antibody-compound combinations, uniformly. Each assay plate was self-anchored containing duplicate 11 × 11 combination matrices, vehicle/DMSO or vehicle/PBS negative controls, cidal positive controls, and the static control agent cycloheximide.

Measurement of Anti-proliferative Activity of Treated Cells

[0101] Cell proliferation assays were performed 72-144 h post-treatment using CellTiter-Glo® Luminescent Cell Viability Assay reagent (Promega) according to the manufacturer's instructions (CellTiter-Glo® Luminescent Cell Viability Assay Technical Bulletin Instructions for Use of Product(s) G7570, G7571, G7572, G7573 Literature # TB288, Revised 3/15), and then measuring the luminescent signal on a microtiter plate reader (Envision, PE).

[0102] Cell proliferation was evaluated using the time zero (T0) signal as the positive control and the within-plate vehicle wells (DMSO) as the negative control. Data was converted to percent inhibition and falls into the range from 0%

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to 100% of growth where 0% equals the signal at TO and 100% equals uninhibited or maximal growth. Cell growth at or near 0% is considered a static response.

[0103] Cell viability or death was evaluated using the response data for within-plate cidal controls compounds (1 μ M bortezomib, 1 μ M staurosporine) and the TO signal as the negative control. Data was converted to percent inhibition and falls into the range from -100% to 0%. Cell growth at or near -100% is considered a cidal response.

Determination of Compound Synergy in vitro

[0104] Compound 1 and the EGFR inhibitors were tested as single-agents and in combinations against the Hep3B cell model. Relative percent inhibition data were calculated by data analysis software (ECABIA, H3 Biomedicine) as described, and then transformed into a 0-200% data format (200% = cidal, 100% = static, 0% = no effect) compatible with further analysis.

[0105] Combination effects were then assessed using Chalice software (Horizon Discovery) comparing combination responses to their matched single-agent effects using the Loewe Additivity Model (Lehar J et al 2009 and Zimmermann GR et al 2006). Drug concentration ranges where synergistic effects occurred can be visualized in Chalice by comparing the full Dose-matrix Chart to the Loewe Additivity Model Chart, and by direct observation of the Excess Response Chart. Quantitative assessment can be made within a study or across anchored studies by the area and intensity of the combination response which is provided by the Chalice synergy score. Self-cross experiments and tests with other additive-only combinations served as baseline controls.

Xenograft Generation, Dosing and Measurement of Antitumor Activity

[0106] The human hepatocellular cancer cell line Hep3B was cultured in RPMI1640 medium containing 10% fetal bovine serum at 37 °C in a 5% CO₂ atmosphere and kept in exponential growth phase. For harvesting, the cells were washed with phosphate buffered saline, incubated with 0.25% trypsin-EDTA, and suspended in a 1:1 mixture of RPMI1640 medium and Matrigel (Corning) at a final concentration of 5 × 10⁷ cells/mL. To generate xenografts, 0.1-mL of the inoculum was injected subcutaneously into the right flank region of mice, giving a final concentration of 5 × 10⁶ cells/mouse. When the mean tumor volume (TV) reached approximately 170 mm³ (10 days after implantation), 56 mice were selected based on their TVs, and were sorted into 7 treatment groups with 8 animals per group. Per os (PO) treatment with Compound 1 (100 and 300 mg/kg) alone or in combination with vehicle (control) or gefitinib (50, 100, 200mg/kg) administered every 12 hours (BID) continued for 15 days. The administration volume (0.1 mL/10 g body weight) was calculated from the individual mouse body weight (BW) before administration. Body weights were measured daily and tumor measurements were performed twice weekly.

[0107] The TV in mm³ was calculated according to the following formula: TV = length × width² × 0.5 length: largest diameter of tumor (mm) width: diameter perpendicular to length (mm) The Tumor Growth Inhibition% (TGI) was calculated according to the following formula:

$$\frac{\text{Average Control TV Day X} - \text{Treatment TV Day X}}{\text{Average Control TV Day X}} \times 100$$

where Day X is any day of measurement.

[0108] The anti-tumor effects of the treatment, Partial (PR) and complete regression (CR), stable (SD) and progressive (PD) disease were defined by the H3 Xenograft Model Response Criteria (Appendix 4). Mice with > 20% body weight loss compared to their Day 1 body weight or bearing tumors with the longest diameter > 2000 mm were immediately euthanized to prevent any pain or suffering in the animal as according to IACUC guidelines defined by the H3 Biomedicine Animal Care and Use Program and study protocol 13-05-1g.

Statistical Analysis

[0109] Data are expressed as the mean ± SEM for TV or ± STDEV for BW. The differences in TV on Day 15 between the vehicle treated and Compound 1 combination treated groups were analyzed by two way ANOVA followed by Dunnett's post hoc test. The relative body weight changes were analyzed by two way ANOVA followed by Dunnett's post hoc. Statistical analyses were performed using the GraphPad Prism version 5.04 (GraphPad Software, La Jolla, CA).

H3 Xenograft Model Response Criteria

[0110] Progressive disease (PD): 3 consecutive measurements >120% of starting volume or 3 consecutive increasing measurements from best response, Stable disease (SD): 3 consecutive measurements >50% and <120% of starting volume, Partial regression (PR): 3 consecutive measurements <50% of starting volume, Complete regression (CR): 3 consecutive measurements <30mm³.

Results

In vitro combination studies

[0111] To determine whether FGFR4 and EGFR inhibitors synergize in reducing cell viability and/or proliferation, Compound 1 (an FGFR4 inhibitor) and four EGFR inhibitors, gefitinib, afatinib, lapatinib, and cetuximab were tested in combinations using HEP3B cell line in vitro (a representative HCC cell line). The cells were exposed to compounds and then the effect on viability was measured after 72 hours.

Compound 1 and Gefitinib

[0112] As single agents, the highest doses of 1 μ M Compound 1 and 5 μ M gefitinib reduced cell viability 72% and 43%, respectively (FIG. 1A) whereas the combination of 1 μ M Compound 1 and 5 μ M gefitinib led to total cell killing (i.e. 200% effect). In addition, lower doses of Compound 1 and gefitinib when combined, reduced cell viability to a greater extent compared to the corresponding single-agent doses (FIG. 1A). Excess inhibition over additivity was calculated using the Loewe Additivity Model and synergistic values were observed starting from 0.01 μ M Compound 1 and 0.05 μ M gefitinib (FIG. 1B).

[0113] FIG. 1A and FIG. 1B show that Compound 1 and gefitinib synergistically inhibit growth of HEP3B cells in vitro. HEP3B cells were treated for 72 hours with different doses of Compound 1 and gefitinib. Inhibition of cell viability was measured using CellTiter-Glo (FIG. 1A), and Chalice software was used to calculate excess inhibition over Loewe additivity for each Compound 1 and gefitinib dose combination (FIG. 1B).

Compound 1 and Afatinib

[0114] As single-agents, the highest doses of 1 μ M Compound 1 and 1 μ M afatinib reduced cell viability 82% and 47%, respectively (FIG. 2A) whereas the combination of 1 μ M Compound 1 and 1 μ M afatinib led to total cell killing (i.e. 200% effect). In addition, lower doses of Compound 1 and afatinib when combined, reduced cell viability to a greater extent compared to the corresponding single agent doses (FIG. 2A). Excess inhibition over additivity was calculated using the Loewe additivity model and synergistic values were observed starting from 0.12 μ M Compound 1 and 0.004 μ M afatinib (FIG. 2B).

[0115] FIG. 2A and FIG. 2B show Compound 1 and afatinib synergistically inhibit growth of HEP3B cells in vitro. HEP3B cells were treated for 72 hours with different doses of Compound 1 and afatinib. Inhibition of cell viability was measured using CellTiter-Glo (FIG. 2A), and Chalice software was used to calculate excess inhibition over Loewe additivity for each Compound 1 and afatinib dose combination (FIG. 2B).

Compound 1 and Lapatinib

[0116] As single agents, the highest doses of 1 μ M Compound 1 and 5 μ M lapatinib reduced cell viability 66% and 61%, respectively (FIG. 3A) whereas the combination of 1 μ M Compound 1 and 5 μ M lapatinib led to total cell killing (i.e. 200% effect). In addition, lower doses of Compound 1 and lapatinib when combined, reduced cell viability to a greater extent compared to the corresponding single agent doses (FIG. 3A). Excess inhibition over additivity was calculated using the Loewe additivity model and synergistic values were observed starting from 0.001 μ M Compound 1 and 0.005 μ M lapatinib (FIG. 3B).

[0117] FIG. 3A and FIG. 3B shows Compound 1 and lapatinib synergistically inhibit growth of HEP3B cells in vitro. HEP3B cells were treated for 72 hours with different doses of Compound 1 and lapatinib. Inhibition of cell viability was measured using CellTiter-Glo (FIG. 3A), and Chalice software was used to calculate excess inhibition over Loewe additivity for each Compound 1 and lapatinib dose combination (FIG. 3B).

Compound 1 and Cetuximab

[0118] As single agents, the highest doses of 1 μ M Compound 1 and 30 nM cetuximab reduced cell viability 46% and

1%, respectively (FIG. 4A) whereas the combination of 1 μ M Compound 1 and 30 nM cetuximab reduced viability greatly (173% effect). In addition, lower doses of Compound 1 and cetuximab when combined, reduced cell viability to a greater extent compared to the corresponding single agent doses (FIG. 4A). Excess inhibition over additivity was calculated using the Loewe additivity model and synergistic values were observed starting from 0.001 μ M Compound 1 and 0.00004 μ M cetuximab (FIG. 4B).

[0119] FIG. 4A and FIG. 4B show Compound 1 and cetuximab synergistically inhibit growth of HEP3B cells in vitro. HEP3B cells were treated for 72 hours with different doses of Compound 1 and cetuximab. Inhibition of cell viability was measured using CellTiter-Glo (FIG. 4A), and Chalice software was used to calculate excess inhibition over Loewe additivity for each Compound 1 and cetuximab dose combination (FIG. 4B).

In vivo combination studies

[0120] To determine whether Compound 1 and gefitinib synergistically affect the tumor growth in vivo, the HEP3B cell line was grown as a xenograft in female nude immunocompromised mice. Tumor-bearing mice were treated daily for 17 days with 100 mg/kg or 300 mg/kg Compound 1, and 50, 100, or 200 mg/kg gefitinib as single agents or in combination. Gefitinib as single agent did not inhibit tumor growth at any dose level (FIG. 5A). In contrast, Compound 1 single agent resulted in Tumor Growth Inhibition (TGI) of 16% at 100mg/kg and 64% at 300mg/kg ($p < 0.05$ for 300 mg/kg Compound 1 compared to vehicle control, FIG. 5B and FIG. 5C).

[0121] The combination of 100mg/kg Compound 1 and gefitinib resulted in significant enhancement of the antitumor effects with all 3 dose levels in comparison to the Compound 1 100mg/kg single agent with TGI of 27% for 50mg/kg (8/8 PD), 32% for 100mg/kg (8/8 PD) and, 72% for 200mg/kg (3/8 SD and 5/8 PD; $p < 0.05$ for all dose combinations using 100 mg/kg Compound 1 compared to vehicle control). The combination of 300mg/kg Compound 1 and gefitinib also resulted in significant enhancement of the antitumor effects with all 3 dose levels in comparison to the Compound 1 300mg/kg single agent with TGI of 75% for 50mg/kg (2/8 SD and 6/8 PD), 89% for 100mg/kg (3/8 PR, 4/8 SD and 1/8 PD), and 98% for 200mg/kg (5/8 CR, 2/8 PR, and 1/8 SD; $p < 0.05$ for all dose combinations for 300 mg/kg Compound 1 compared to vehicle control). None of the treatments resulted in any toxicities as measured by body weight or observations of pre-clinical health conditions (data not shown).

[0122] FIG. 5A, FIG. 5B, and FIG. 5C show antitumor effects of oral Compound 1 and gefitinib in the Hep3B xenograft model of hepatocellular carcinoma. Both Compound 1 and gefitinib was given orally (PO) once daily (QD) for 17 days. Data represent the mean \pm SEM (Tumor Volume). FIG. 5A shows gefitinib as single agent. FIG. 5B shows three doses of gefitinib with 100mg/kg *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof (* $p < 0.05$ at the 100 mg/kg Compound 1 dose in combination with 200 mg/kg gefitinib compared to 100 mg/kg Compound 1 single agent using two way ANOVA followed by Dunnett's post hoc test). FIG. 5C shows three doses of gefitinib with 300mg/kg Compound 1 (* $p < 0.05$ at the 300 mg/kg dose of Compound 1 in combination with 100 or 200 mg/kg gefitinib compared to 300 mg/kg Compound 1 using two way ANOVA followed by Dunnett's post hoc test).

Claims

1. A combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof for use in a method of treating hepatocellular carcinoma in a patient in need thereof.
2. The combination for use according to claim 1, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof is administered in a daily dosage between 50 mg to 600 mg, optionally in a daily dosage between 200 mg to 400 mg, or further optionally in a daily dosage of 300 mg.
3. The combination for use according to any one of claims 1-2, wherein the EGFR inhibitor is selected from the group consisting of 4-quinazolinamine *N*-(3-chloro-4-fluorophenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy] ("gefitinib"), *N*-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[(3*S*)-tetrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(dimethylamino)-2-butenamide ("afatinib"), *N*(3-chloro-4-[[3-fluorophenyl)methyl]oxy]phenyl)-6-[5-[[2-(methylsulfonyl)ethyl]amino)methyl]-2-furanyl]-4-quinazolinamine bis(4-methylbenzenesulfonate) monohydrate ("lapatinib"), and cetuximab.
4. The combination for use according to claim 3, wherein the EGFR inhibitor is gefitinib, optionally wherein the gefitinib is administered in a daily dosage of 250 mg.

5. The combination for use according to claim 3, wherein the EGFR inhibitor is afatinib, optionally wherein the afatinib is administered in a daily dosage of 20 mg/day, or optionally wherein the afatinib is administered in a daily dosage of 30 mg/day, or optionally wherein the afatinib is administered in a daily dosage of 40 mg/day .
6. The combination for use according to claim 3, wherein the EGFR inhibitor is lapatinib, optionally wherein the lapatinib is administered in a daily dosage of between 1000 mg to 1500 mg, or optionally wherein the lapatinib is administered in a daily dosage of 1000 mg, or optionally wherein the lapatinib is administered in a daily dosage of 1250 mg, or optionally wherein the lapatinib is administered in a daily dosage of 1500 mg.
7. The combination for use according to claim 3, wherein the EGFR inhibitor is cetuximab, optionally wherein the cetuximab is administered in a weekly dosage of between 200-300 mg/m², or optionally wherein the cetuximab is administered in a weekly dosage of 250 mg/m².
8. The combination for use according to any one of claims 1-7, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are separate formulations administered simultaneously.
9. The combination for use according to any one of claims 1-7, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are administered as a single formulation.
10. The combination for use according to any one of claims 1-7, wherein the *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and the EGFR inhibitor are separate formulations administered sequentially..
11. The combination for use according to any one of claims 1-10, comprising the free base form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.
12. The combination for use according to any one of claims 1-10, wherein the pharmaceutically acceptable salt of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a hydrochloride salt form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.
13. A pharmaceutical formulation comprising *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof.
14. The pharmaceutical formulation of claim 13, comprising a free base form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.
15. The pharmaceutical formulation of claim 13, wherein the pharmaceutically acceptable salt of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide is a hydrochloride salt form of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide.
16. A combination of *N*-(2-((6-(3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamide or a pharmaceutically acceptable salt thereof and an EGFR inhibitor or a pharmaceutically acceptable salt thereof for use in a method of reducing hepatocellular carcinoma cell viability or hepatocellular carcinoma cell proliferation by administering the combination to one or more cells.

Patentansprüche

1. Kombination von *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder einem pharmazeutisch unbedenklichen Salz davon und einem EGFR-Inhibitor oder einem pharmazeutisch unbedenklichen Salz davon zur Verwendung bei einem Verfahren zur Behandlung von Leberzellkarzinom bei einem Patienten, bei dem diesbezüglicher Bedarf besteht.

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2. Kombination zur Verwendung nach Anspruch 1, wobei das *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder ein pharmazeutisch unbedenkliches Salz davon in einer Tagesdosis zwischen 50 mg bis 600 mg, gegebenenfalls in einer Tagesdosis zwischen 200 mg bis 400 mg oder ferner gegebenenfalls in einer Tagesdosis von 300 mg verabreicht wird.
3. Kombination zur Verwendung nach einem der Ansprüche 1-2, wobei der EGFR-Inhibitor aus der Gruppe bestehend aus 4-Chinazolinamin-*N*-(3-chlor-4-fluorphenyl)-7-methoxy-6-[3-(4-morpholinyl)propoxy] ("Gefitinib"), *N*-[4-[(3-Chlor-4-fluorphenyl)amino]-7-[(3*S*)-tetrahydro-3-furanyl]oxy]-6-chinazolinyl]-4(dimethylamino)-2-butenamid ("Afatinib"), *N*-(3-Chlor-4-[[[(3-fluorphenyl)methyl]oxy]phenyl]-6-[5-([2-(methylsulfonyl)ethyl]amino)methyl]-2-furanyl]-4-chinazolinaminbis(4-methylbenzolsulfonat)monohydrat ("Lapatinib") und Cetuximab ausgewählt ist.
4. Kombination zur Verwendung nach Anspruch 3, wobei es sich bei dem EGFR-Inhibitor um Gefitinib handelt, gegebenenfalls wobei das Gefitinib in einer Tagesdosis von 250 mg verabreicht wird.
5. Kombination zur Verwendung nach Anspruch 3, wobei es sich bei dem EGFR-Inhibitor um Afatinib handelt, gegebenenfalls wobei das Afatinib in einer Tagesdosis von 20 mg/Tag verabreicht wird oder gegebenenfalls wobei das Afatinib in einer Tagesdosis von 30 mg/Tag verabreicht wird oder gegebenenfalls wobei das Afatinib in einer Tagesdosis von 40 mg/Tag verabreicht wird.
6. Kombination zur Verwendung nach Anspruch 3, wobei es sich bei dem EGFR-Inhibitor um Lapatinib handelt, gegebenenfalls wobei das Lapatinib in einer Tagesdosis zwischen 1000 mg bis 1500 mg verabreicht wird oder gegebenenfalls wobei das Lapatinib in einer Tagesdosis von 1000 mg verabreicht wird oder gegebenenfalls wobei das Lapatinib in einer Tagesdosis von 1250 mg verabreicht wird oder gegebenenfalls wobei das Lapatinib in einer Tagesdosis von 1500 mg verabreicht wird.
7. Kombination zur Verwendung nach Anspruch 3, wobei es sich bei dem EGFR-Inhibitor um Cetuximab handelt, gegebenenfalls wobei das Cetuximab in einer Wochendosis zwischen 20-300 mg/m² verabreicht wird oder gegebenenfalls wobei das Cetuximab in einer Wochendosis von 250 mg/m² verabreicht wird.
8. Kombination zur Verwendung nach einem der Ansprüche 1-7, wobei es sich bei dem *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder einem pharmazeutisch unbedenklichen Salz davon und dem EGFR-Inhibitor um gleichzeitig verabreichte separate Formulierungen handelt.
9. Kombination zur Verwendung nach einem der Ansprüche 1-7, wobei das *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder ein pharmazeutisch unbedenkliches Salz davon und der EGFR-Inhibitor in Form einer einzigen Formulierung verabreicht werden.
10. Kombination zur Verwendung nach einem der Ansprüche 1-7, wobei es sich bei dem *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder einem pharmazeutisch unbedenklichen Salz davon und dem EGFR-Inhibitor um aufeinanderfolgend verabreichte separate Formulierungen handelt.
11. Kombination zur Verwendung nach einem der Ansprüche 1 bis 10, umfassend die freie Basenform von *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid.
12. Kombination zur Verwendung nach einem der Ansprüche 1-10, wobei es sich bei dem pharmazeutisch unbedenklichen Salz von *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid um eine Hydrochloridsalzform von *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid handelt.
13. Pharmazeutische Formulierung, umfassend *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder ein pharmazeutisch unbedenkliches Salz davon und einen EGFR-Inhibitor oder ein pharmazeutisch unbedenkliches Salz davon.
14. Pharmazeutische Formulierung nach Anspruch 13, umfassend die freie Basenform von *N*-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid.

15. Pharmazeutische Formulierung nach Anspruch 13, wobei es sich bei dem pharmazeutisch unbedenklichen Salz von N-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid um eine Hydrochloridsalzform von N-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid handelt.

16. Kombination von N-(2-((6-(3-(2,6-Dichlor-3,5-dimethoxyphenyl)-1-methylureido)pyrimidin-4-yl)amino)-5-(4-ethylpiperazin-1-yl)phenyl)acrylamid oder einem pharmazeutisch unbedenklichen Salz davon und einem EGFR-Inhibitor oder einem pharmazeutisch unbedenklichen Salz davon zur Verwendung bei einem Verfahren zur Verringerung der Lebensfähigkeit von Leberzellkarzinomzellen oder Proliferation von Leberzellkarzinomzellen durch Verabreichung der Kombination an eine oder mehrere Zellen.

Revendications

1. Combinaison de N-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou d'un sel pharmaceutiquement acceptable correspondant et d'un inhibiteur d'EGFR ou d'un sel pharmaceutiquement acceptable correspondant pour une utilisation dans un procédé de traitement d'un carcinome hépatocellulaire chez un patient qui en a besoin.

2. Combinaison pour une utilisation selon la revendication 1, le N-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou un sel pharmaceutiquement acceptable correspondant étant administrée en un dosage quotidien compris entre 50 mg et 600 mg, éventuellement en un dosage quotidien compris entre 200 mg et 400 mg ou en outre éventuellement en un dosage quotidien de 300 mg.

3. Combinaison pour une utilisation selon l'une quelconque des revendications 1 et 2, l'inhibiteur d'EGFR étant choisi dans le groupe constitué par la N-(3-chloro-4-fluorophényl)-7-méthoxy-6-[3-(4-morpholanyl)propoxy]-4-quinazolinamine (« géfitinib »), le N-[4-[(3-Chloro-4-fluorophényl)amino]-7-[[3S)-tétrahydro-3-furanyl]oxy]-6-quinazolinyl]-4(diméthylamino)-2-buténamide (« afatinib »), le monohydrate de bis(4-méthylbenzènesulfonate) de N-(3-chloro-4-[[3-fluorophényl)méthyl]oxy]phényl)-6-[5-[[2-(méthylsulfonyl)éthyl]amino)méthyl]-2-furannyl]-4-quinazolinamine (« lapatinib »), et le cétuximab.

4. Combinaison pour une utilisation selon la revendication 3, l'inhibiteur d'EGFR étant le géfitinib, éventuellement le géfitinib étant administré en un dosage quotidien de 250 mg.

5. Combinaison pour une utilisation selon la revendication 3, l'inhibiteur d'EGFR étant l'afatinib, éventuellement l'afatinib étant administré en un dosage quotidien de 20 mg/jour, ou éventuellement l'afatinib étant administré en un dosage quotidien de 30 mg/jour, ou éventuellement l'afatinib étant administré en un dosage quotidien de 40 mg/jour.

6. Combinaison pour une utilisation selon la revendication 3, l'inhibiteur d'EGFR étant le lapatinib, éventuellement le lapatinib étant administré en un dosage quotidien compris entre 1 000 mg et 1 500 mg, ou éventuellement le lapatinib étant administré en un dosage quotidien de 1 000 mg, ou éventuellement le lapatinib étant administré en un dosage quotidien de 1 250 mg, ou éventuellement le lapatinib étant administré en un dosage quotidien de 1 500 mg.

7. Combinaison pour une utilisation selon la revendication 3, l'inhibiteur d'EGFR étant le cétuximab, éventuellement le cétuximab étant administré en un dosage hebdomadaire compris entre 200 et 300 mg/m², ou éventuellement le cétuximab étant administré en un dosage hebdomadaire de 250 mg/m².

8. Combinaison pour une utilisation selon l'une quelconque des revendications 1 à 7, le N-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou un sel pharmaceutiquement acceptable correspondant et l'inhibiteur d'EGFR étant des formulations distinctes administrées simultanément.

9. Combinaison pour une utilisation selon l'une quelconque des revendications 1 à 7, le N-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou un sel pharmaceutiquement acceptable correspondant et l'inhibiteur d'EGFR étant administrés en tant que formulation unique.

10. Combinaison pour une utilisation selon l'une quelconque des revendications 1 à 7, le N-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou un sel phar-

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maceutiquement acceptable correspondant et l'inhibiteur d'EGFR étant des formulations distinctes administrées séquentiellement.

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11. Combinaison pour une utilisation selon l'une quelconque des revendications 1 à 10, comprenant la forme de base libre du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide.
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12. Combinaison pour une utilisation selon l'une quelconque des revendications 1 à 10, le sel pharmaceutiquement acceptable du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide étant une forme de sel de chlorhydrate du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide.
- 15
13. Formulation pharmaceutique comprenant du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou un sel pharmaceutiquement acceptable correspondant et un inhibiteur d'EGFR ou un sel pharmaceutiquement acceptable correspondant.
- 20
14. Formulation pharmaceutique selon la revendication 13, comprenant une forme de base libre du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide.
- 25
15. Formulation pharmaceutique selon la revendication 13, le sel pharmaceutiquement acceptable du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide étant une forme de sel de chlorhydrate du *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide.
- 30
16. Combinaison de *N*-(2-((6-(3-(2,6-dichloro-3,5-diméthoxyphényl)-1-méthyluréido)pyrimidin-4-yl)amino)-5-(4-éthylpipérazin-1-yl)phényl)acrylamide ou d'un sel pharmaceutiquement acceptable correspondant et d'un inhibiteur d'EGFR ou d'un sel pharmaceutiquement acceptable correspondant pour une utilisation dans un procédé de réduction de la viabilité cellulaire d'un carcinome hépatocellulaire ou de la prolifération cellulaire d'un carcinome hépatocellulaire par administration de la combinaison à une ou plusieurs cellules.

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FIG. 1A

DOSE MATRIX

5	43	41	41	43	35	51	104	163	194	196	199	200
	12	20	19	21	16	25	74	109	181	185	190	198
.5	8	8	2	18	15	20	64	86	160	166	179	188
	10	18	6	9	16	16	55	69	97	106	112	116
.05	-3	9	7	11	5	8	40	53	77	82	74	84
	4	6	10	6	5	11	36	47	66	71	64	68
5e-3	7	5	9	-5	13	12	39	38	66	63	62	57
	16	-2	3	8	13	6	25	35	57	61	58	65
5e-4	3	9	1	-5	7	6	23	39	53	59	54	63
	8	-3	8	9	8	7	32	39	49	55	63	56
5e-5	8	0	-5	5	11	6	29	36	50	56	53	59
0	-	10	11	4	16	22	36	51	60	66	61	72
	0	1e-5	1e-4	1e-3	.01	.1	1					

Vertical axis is Gefitinib (μM).

Horizontal axis is Compound 1 (μM).

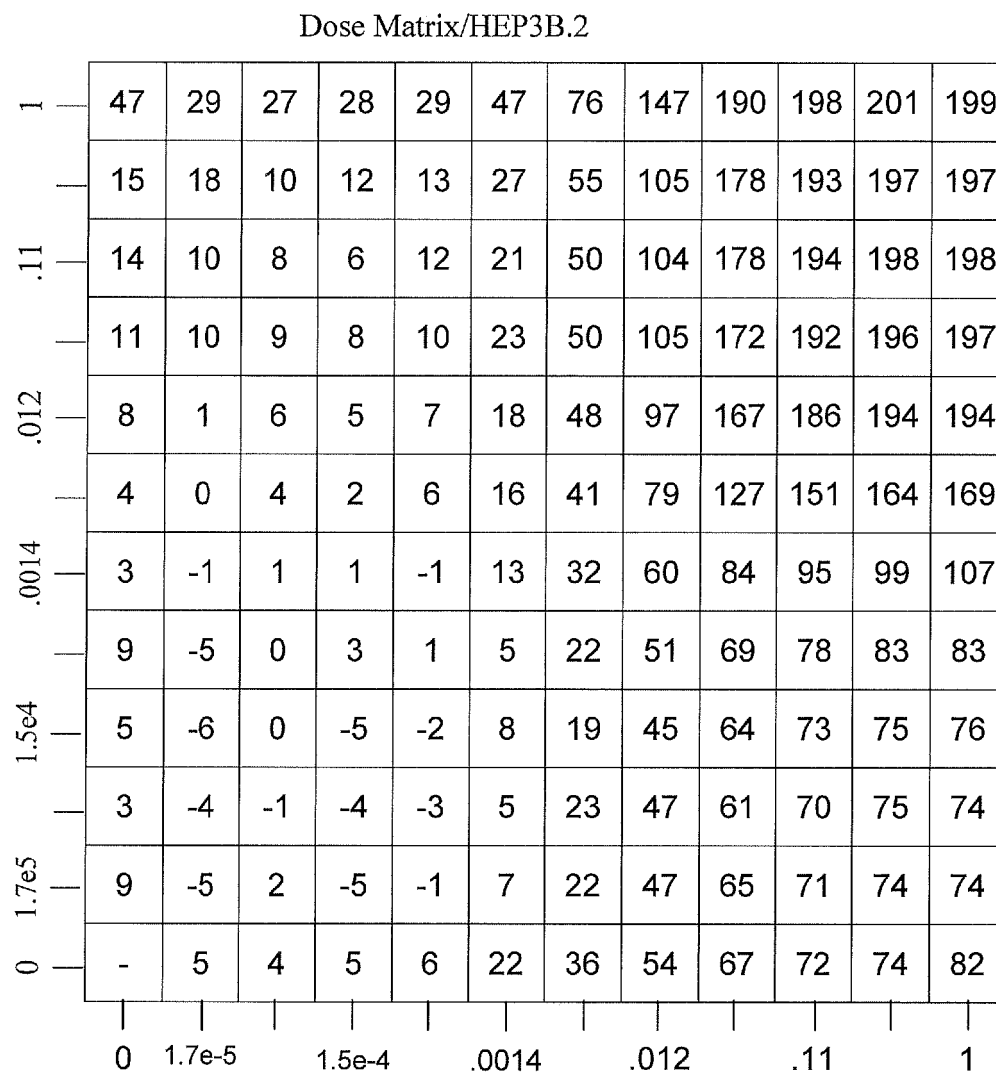
FIG. 1B

Loewe Excess over Additivity

5	1	-2	-2	0	-8	7	59	113	134	131	131	131
	-1	6	4	3	-8	-7	32	59	121	120	122	129
.5	7	5	-3	9	-1	-6	25	36	100	101	111	119
	9	16	3	1	2	-9	17	18	37	41	44	47
.05	-3	7	4	4	-9	-16	3	3	17	17	6	15
	4	5	7	0	-8	-13	-1	-3	6	6	-4	-1
5e-3	6	4	6	-11	0	-12	2	-12	6	-2	-6	-12
	15	-3	0	2	-1	-18	-13	-15	-2	-4	-10	-4
5e-4	3	8	-2	-11	-7	-18	-15	-11	-6	-6	-14	-6
	7	-4	4	3	-6	-17	-5	-12	-11	-10	-5	-13
5e-5	7	-1	-8	-2	-2	-18	-9	-14	-10	-8	-15	-10
0	-	9	8	-3	3	-2	-2	0	0	1	-7	3
	0	1e-5	1e-4	1e-3	1e-3	1e-3	.01	.01	.1	.1	1	1

Vertical axis is Gefitinib (μM).Horizontal axis is Compound 1 (μM).

FIG. 2A



Vertical axis is Afatinib (μM).

Horizontal axis is Compound 1 (μM).

FIG. 2B

Loewe Excess over Additivity

1	0	-17	-20	-19	-17	0	29	92	123	126	126	117
	0	3	-7	-7	-12	-5	12	51	111	122	123	115
.11	0	-4	-6	-8	-2	-5	11	49	111	122	123	116
	0	-1	-2	-4	-3	0	13	50	105	120	121	115
.012	0	-8	-3	-4	-4	-5	12	43	100	114	119	112
	0	-9	-5	-7	-3	-6	5	24	60	79	90	87
.0014	0	-10	-8	-9	-10	-9	-4	6	17	24	25	25
	0	-14	-9	-6	-8	-17	-14	-4	2	7	9	1
1.5e4	0	-15	-9	-14	-11	-14	-17	-10	-2	1	0	-6
	0	-13	-10	-13	-12	-17	-13	-8	-6	-2	1	-8
1.7e5	0	-14	-7	-13	-10	-15	-14	-7	-2	-1	-1	-8
0	-	0	0	0	0	0	0	0	0	0	0	0
	0	1.7e-5	1.5e-4	.0014	.012	.11	1					

Verticle axis is Afatinib (uM).

Horizontal axis is Compound 1 (uM).

FIG. 3A

Dose Matrix / HEP3B.2

5	61	67	72	68	78	81	179	190	192	198	199	200
	24	37	35	38	43	42	104	158	187	188	196	198
.5	17	14	8	9	21	28	83	105	161	172	183	190
	13	6	0	17	16	11	67	86	127	138	145	156
.05	6	10	16	9	18	18	59	79	98	107	120	132
	14	7	11	12	12	10	45	67	75	82	91	93
5e-3	4	10	10	22	14	12	47	49	59	77	72	74
	12	-1	12	11	9	10	37	44	45	66	64	67
5e-4	5	3	1	-3	0	3	38	48	53	58	65	58
	-4	-13	5	7	12	2	37	37	46	65	61	54
5e-5	5	-5	-2	10	13	7	36	41	47	61	59	57
0	-	4	15	6	8	14	44	51	59	62	64	66
	0	1e-5	1e-4	1e-3	.01	.1	1					

Verticle axis is Lapatinib (uM).

Horizontal axis is Compound 1 (uM).

FIG. 3B

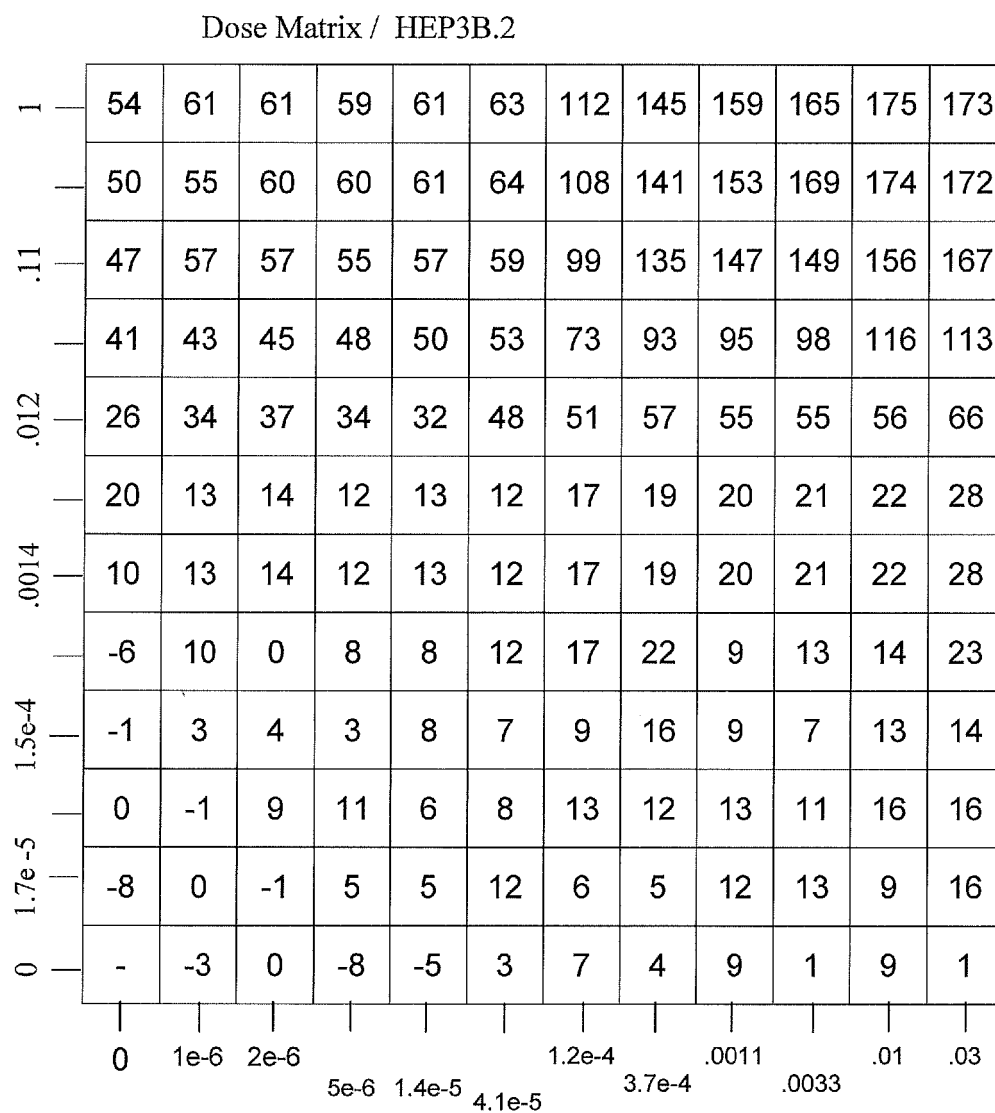
Loewe Excess over Additivity

5	7	13	18	14	24	26	123	132	132	137	138	139
	-12	1	-2	1	4	0	56	103	128	128	135	137
.5	2	-1	-8	-8	-1	-1	40	51	102	112	122	129
	9	2	-5	11	4	-12	27	32	58	78	s84	95
.06	5	9	14	5	10	-3	19	25	39	47	59	71
	14	7	10	10	5	-10	6	13	16	22	30	32
5e-3	3	9	9	20	7	-8	8	-4	0	17	11	13
	11	-1	11	9	2	-10	-2	-10	-13	6	3	6
5e-4	5	2	0	-6	-7	-17	-1	-6	-6	-2	4	-3
	-5	-13	4	5	5	-18	-2	-16	-13	5	0	-7
5e-5	5	-6	-3	7	6	-13	-3	-12	-12	1	-2	-3
0	-	4	14	4	1	-6	5	-2	0	2	3	5
	0	1e-5	1e-4	1e-3	1e-3	1e-3	.01	.01	.01	.1	.1	1

Verticle axis is Lapatinib (uM).

Horizontal axis is Compound 1 (uM).

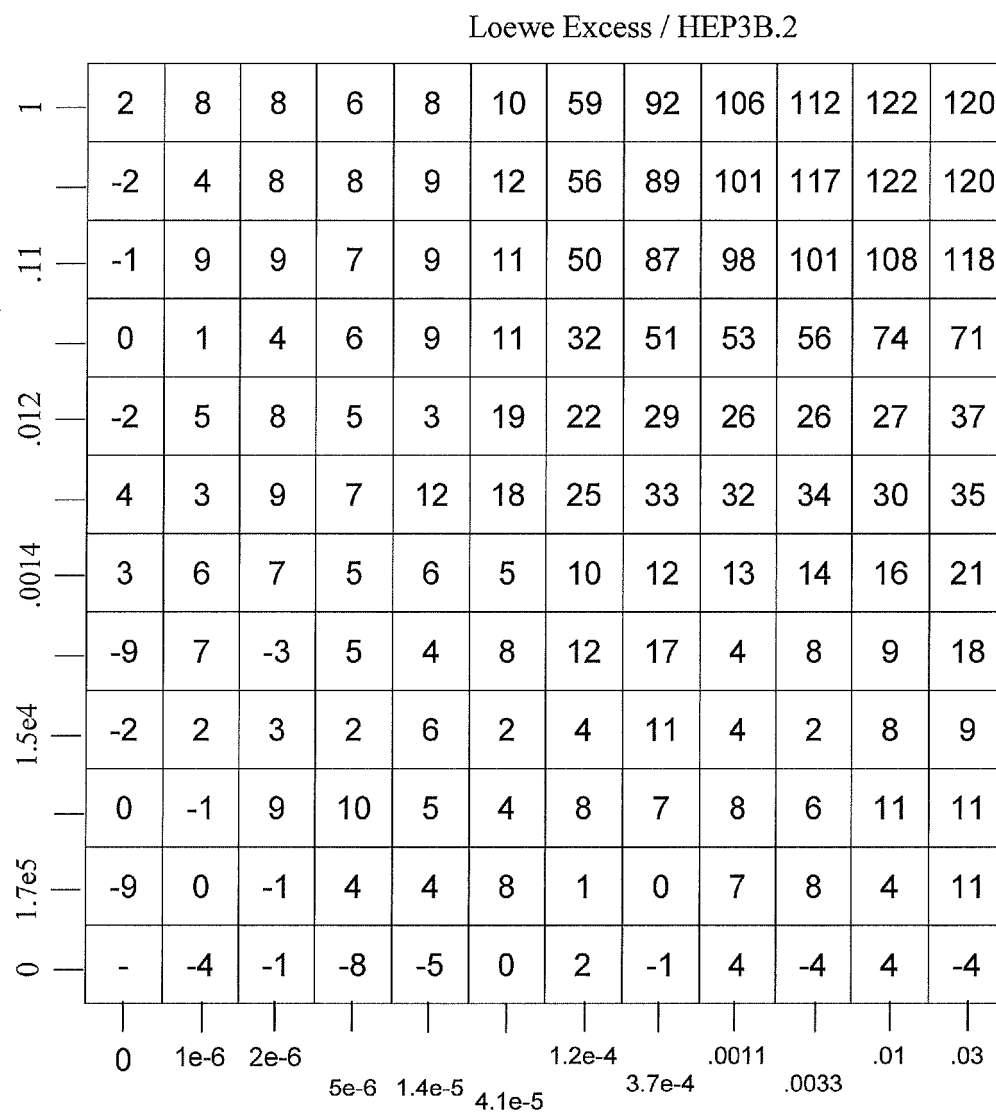
FIG. 4A



Vertical axis is Compound 1 (μM).

Horizontal axis is Cetuximab (μM).

FIG. 4B



Vertical axis is Compound 1 (μM).

Horizontal axis is Cetuximab (μM).

FIG. 5A

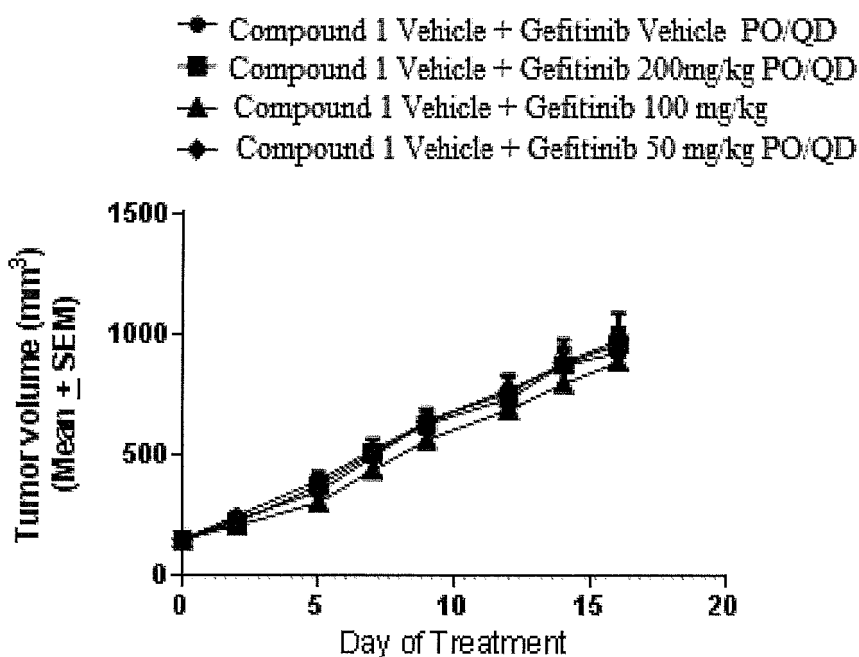


FIG. 5B

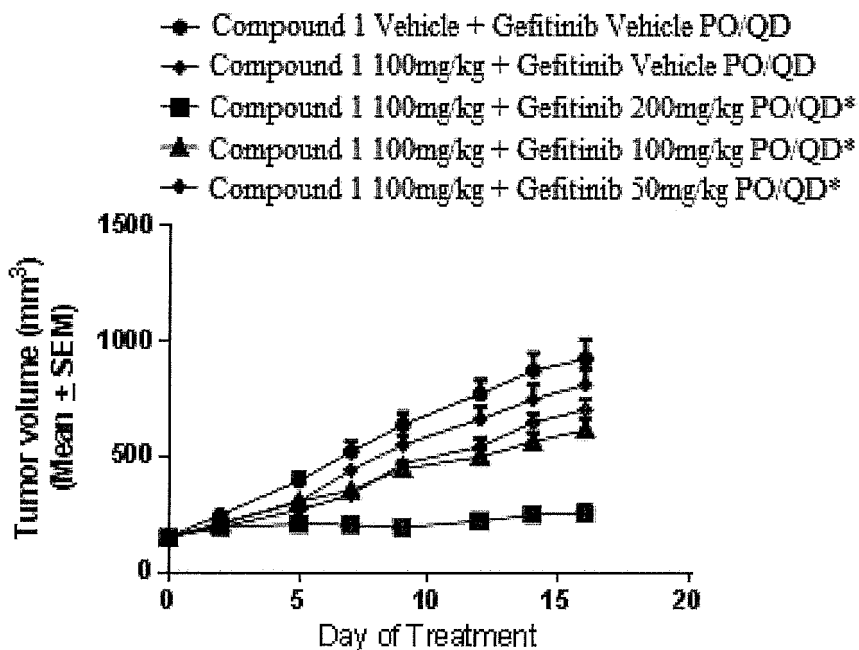
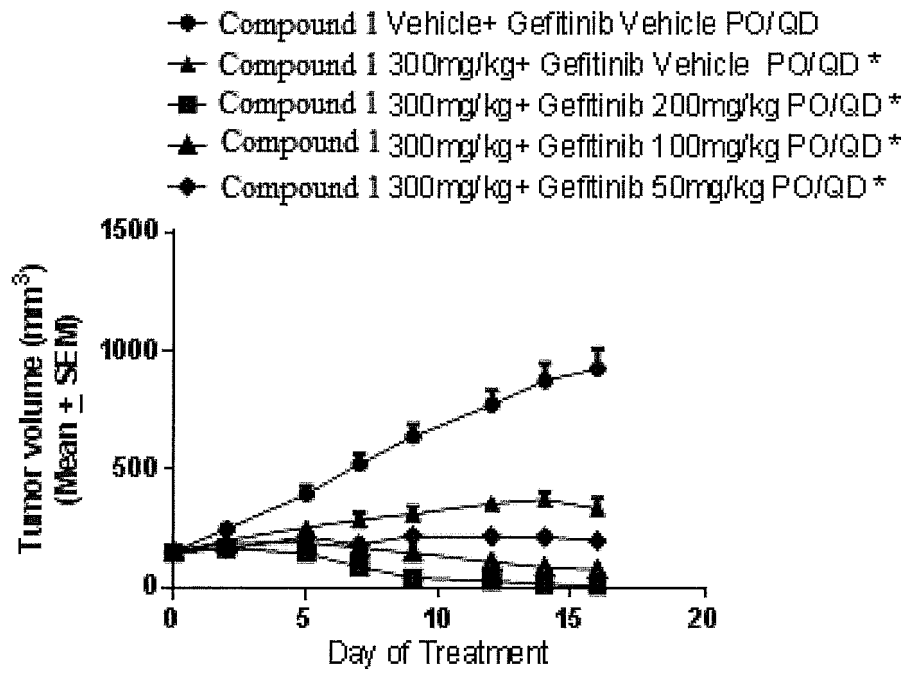


FIG. 5C



REFERENCES CITED IN THE DESCRIPTION

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